EFFICACY REVIEW

Product(s): BL-2003 Mole Bait

Date: March 19, 2004

EPA Reg No(s): 12455-RNR

DP Bar code(s): D298944

Chemical Code: Bromethalin 112802

Formulation(s): Bromethalin gel worm

Purpose for Review: The purpose for this review is to determine if the Bromethalin gel worm is efficacious against the Eastern mole (*Scalopus aquaticus*) and the laboratory and field data collected are acceptable for registration of the above named product.

MRID No(s): **46090006** Jeans, S. N. September 30, 2003. Efficacy of Bromethalin Mole Bait on Wild Caught Eastern Moles. Bell Laboratories, Inc. Unpublished Report. Experiment #BEL/0803/BE522. 88pp.

46153702 Jeans, S. N. December 12, 2003. Field Efficacy of Bromethalin Mole Bait on Established Eastern Mole Populations. Bell Laboratories, Inc. Unpublished Report. Experiment #BEL/0703/BE516. 115pp.

Good Laboratory Practices: Yes

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IRB Reviewer: Geraldine R. McCann, Biologist

BACKGROUND: Bell Laboratories, Inc has applied for a new product registration for their **BL-2003 Mole Bait** (12455-RNR) formulated with the active ingredient Bromethalin. They held a captive population of moles and collected body weight information from July 23, 2002, until July 23, 2003. It was determined that using a diet solely comprised of nightcrawlers fed in excess of daily requirements, exceeds the daily requirements for nutrition and hydration substantiated by the body weight data collected on the captive population. The age of the captured moles for the test was determined by their body weights. According to their reference (Stone and Gorman 1990), a 25-day old mole should weigh about 60 grams. Based on the weight, sex, date of capture, and site trapping history, all the animals were determined to be juveniles post weaning to adults. The efficacy data guideline used to screen the bait for effectiveness for these product is OPP Pesticide Guideline Subdivision G 96-8 Mole Toxicants. This

review will evaluate the results of the laboratory and field studies and determine if the data are acceptable.

REVIEW OF DATA:

1. **46090006** Jeans, S. N. September 30, 2003. Efficacy of Bromethalin Mole Bait on Wild Caught Eastern Moles. Bell Laboratories, Inc. Unpublished Report. Experiment #BEL/0803/BE522. 88pp.

DISCUSSION: This is the first bromethalin product to be used for mole control and the first time data has been submitted on a captive population of moles for efficacy testing. The study guideline reference used is quoted as Environmental Protection Agency, Subdivision F, 40 CFR § 158.340 Guideline Reference Number 96-8 and EPA Good Laboratory Practices, 40 CFR Part 160. The moles in this study (10 males and 10 females) were caught using a Nash Choker Loop Mole Trap modified to snare the moles and minimize mortality in the capture process. All moles were captured in southern Wisconsin, identified as Eastern Moles (Common Moles or *Scalopus aquaticus*), single housed in plastic tubs (10 control and 10 test subjects, 5 of each sex), and offered a subsistence of nightcrawlers *ad libitum* in the pretest holding period. The pretest holding period was 21 days to assure that the test animals were healthy. The pretest acclimation was 3 days.

The test animals were weighed on August 14, 2003. The females averaged 85.68 g and the males 106.62 g. The average difference between the males and females pretest was 20.94 g. The test began August 17, 2003. The OPP guideline 1.209 (Standard Norway Rat/Roof Rat Acute Dry Bait Laboratory Test Method) was referenced as the laboratory standards for the test procedure (page 13 of 88).

The moles were individually housed, subjected to a 12 hour light-dark cycle, kept at 20-25 C°, and the humidity ranged from 30-70 %. They were maintained per Bell's SOP BIO528.1. The test animals received a 2-choice diet of Bromethalin Bait worms and nightcrawlers. The Bromethalin worm was placed in the rear of the plastic tub on the first day of the trial while the nightcrawlers were placed in the rear of the tub. On the second day of the trial, it is not noted where the toxic worm or the nightcrawlers were placed. In the guidelines, it is specified that the bait and control diet should be switched to deter feeding preference. Also, in the guidelines it states in 96-8 (c): The purpose of these studies is to assess the appropriateness of bait carriers used in subsequent testing. I interpret this to mean that the control animals should have been given 2 choices as well: natural diet (earthworms) and fabricated gel-worms.

The composition and formulation of the test baits is unique. It is in the form identified as Bromethalin Mole Bait with the active ingredient bromethalin and appears like a yellow rubber-like solid. Batch number L2116 (0.025 % bromethalin) was used for the correction factor tests (page 71 of 88). To ensure correct food consumption calculations, individual gel worms were weighed and placed in 50 cm^3 mesh canisters filled with the dirt media from the individual test enclosures. The canisters were placed back in the individual enclosures and removed daily at the time of food consumption data collection. The weight gain

or loss in sample weight was used as a factor for correction in the accurate determination of individual daily food consumption for each individual. The male moles received bait batch number L2115, L2116, and L2118 (0.0261 %, 0.025 %, and 0.0263 % bromethalin, respectfully). while the females received bait batch number L2116, L2117, and L2118 (0.025 %, 0.0266 %, and 0.0263 % bromethalin, respectfully). The active ingredient on the label is listed as a 0.025% bromethalin.

During the test period of 2 days, the male and female test animals consumed a total of 176.7 g of the toxic rubber-like bromethalin mole bait (according to the laboratory records). They consumed 1164.8 g of nightcrawlers (according to the laboratory records). The total percent palatablitiy for the test groups is 13.2 % (no standard is listed in either guideline for palatability for moles) with 80 % mortality. The guideline 96-8 (d) (1) states the exposure period for a single dose bait is 3 days and the level of efficacy should be 90 % mortality. No control animals died and the test lasted 11 days.

The test results are summarized below:

Table 1. Captive Eastern Moles on Bromethalin Earthworm Bait

Pretest Weights **1-Day Test-Consumption and Mortality**

Sex	**Average Group Weight (g)**	**Earthworm Diet (g)**	**Toxic Earthworm Bait (g)**	**Total Consumption (g)**
M (5)	102.6	996.3	177.4	1173.7
F (5)	84.62	**80% Mortality**		Percent **Toxic Bait** Consumed **15.1%**
Total (10)	Group Difference 17.98			

Table 1. Control Captive Eastern Moles on Earthworm Diet

Pretest Weights **1-Day Test-Consumption and Mortality**

Sex	**Average Group Weight (g)**	**Earthworm Diet Consumed (g)**	**Total Earthworm Consumption (g)**
M (5)	108.36	1340.9	1340.9
F (5)	86.74	**0% Mortality**	
Total (10)	Group Difference 21.62		

2. **46153702** Jeans, S. N. December 12, 2003. Field Efficacy of Bromethalin Mole Bait on Established Eastern Mole Populations. Bell Laboratories, Inc. Unpublished Report. Experiment #BEL/0703/BE516. 115pp.

DISCUSSION: This study is the field test to accompany the above described laboratory test. All moles were studied in 2 residential southern Wisconsin neighborhoods. The test and control sites exhibited similar agricultural practices. The test and control sites were not in the same area, they were "within relative distance of 70 km" (43.5 miles) from each other. There is no guidance in the OPP Pesticide Guideline Subdivision G 96-8 (Mole Toxicants) for distance of complimentary study areas. The soil samples from the two sites are relatively comparable:

Test Site	% Sand	% Silt	% Clay	pH	% Carbon	Texture
Treated	54	33	13	6.1	0.43	Sandy loam
Control	80	7	13	7.2	0.73	Sandy loam

Both sites were measured, mapped, and monitored according to Bell Laboratories Test Method BIO515.1. Instruments to measure environmental conditions (temperature, humidity, and precipitation) and soil moisture were installed at both locations.

Evidence of mole activity was located on the second day at the sites. To identify occupied mole runs, a "prod stick" with a 2.5 cm (0.98 in) diameter" was used to make holes in the roof of all observed runs at a distance of 1 prod hole/$2m^2$ and identified with a flag at the location. On Day 2, the prod holes were checked for repair or patching termed "sealing" (Mead-Briggs and Woods, 1972) as a measure of activity. The sealed prod holes were deemed active and given a "hole number". New runs were located at the sites and additional prod holes were identified until Day 4 when the "run incorporation" stopped. The treated site had a total of 7 assessment holes with only 2 assessment holes (associated with 2 runs) that satisfied the ≥50% assessment activity criterion specified in the Bell Laboratories Test Method BIO515.1. The control area had 23 assessment holes with 17 assessment holes ("and their associated run systems") that satisfied the ≥50% assessment activity criterion specified in the Bell Laboratories Test Method BIO515.1.

Baiting occurred on Day 8. On the treated site, only the 2 assessment holes and their associated runs remained active. A 1.3 cm (0.51 in) diameter prod was used to make a hole in the roof of the run to deliver the test material. A 5 g Bromethalin Mole Bait gel worm was inserted into the opening and the run was pinched shut to prevent perceivable run disturbance and any entry of light. This methodology was used along the length of the runs at the application rate of 1 gel worm per meter. The 17 assessment holes (and their associated run systems) at the control site were not baited. The assessment holes at both the test and control sites were reopened. The study sites were left fallow on Day 9. On Day 10, the Mole Contact Ratio [(sealed selected runs/selected runs)*100] (MCR) was used to validate the presence of target animals, mole movement, and to establish potential animal exposure to the test material. The treated site experienced a 100% reduction in activity by Day 11 and the control site exhibited a 76.5% activity level 72 hours post baiting.

The composition and formulation of the test baits is unique. It is in the form identified as Bromethalin Mole Bait with the active ingredient bromethalin and appears like a yellow rubber-like solid worm. Batch number I.2113 (0.0221 % bromethalin) was used on the treated site. During storage of the bait, under ambient conditions in polyethylene bags, the bait analysis revealed loss of product stability (bromethalin content dropped to 0.0193 %). Product stability in vacuum-sealed polypropylene bags is being explored in study BEL/0703/C324 Storage Stability and Corrosion Characteristics of Bromethalin Mole Bait. No dates associated with the study were mentioned.

The Bromethalin Mole Bait was applied in a single application at a rate of 1.41 kg/ha [Eighteen (96 g) - 5.3 g worms/2.471 acres] to result in 100 % reduction within 3 days of application. This reduction in activity was maintained throughout the 7-day post test period. The control area (which received no treatment) experienced a 29.4 % reduction in control.

Selection of mole infested sites was made to satisfy criterion to minimize variability associated with environmental influences such as climate, agricultural practices, and soil characteristics. The eastern mole is widely distributed in Wisconsin, but was not trapped out specifically for identification. A mole contact ratio (Mole Contact Ratio) study was incorporated with the baiting study. The test site MCR (50 %) indicated the mole population had potential test material exposure in 50 % if the runs deemed active in the initial run selection phase. And the control site demonstrated a 94.1% MCR indicated that the same methodology could be used to predict mole movement with 94.1 % success.

Efficacy Comments

1. The laboratory testing : The OPP guideline 1.209 (Standard Norway Rat/Roof Rat Acute Dry Bait Laboratory Test Method) was referenced as the laboratory standards for the test procedure (page 13 of 88) and the SOP BIO528.1 was referenced. In future submissions, please provide copies of referenced SOPs used in test procedures so that we may assess their relavance.

2. On the second day of the trial, it is not noted where the toxic worm or the nightcrawlers were placed. Were they switched to deter feeding preference?

3. The mortality of the laboratory efficacy study was 80 % with 13.2 % palatability (no standard is listed in either guideline listed above for palatability to moles). The guideline 96-8 (d) (1) states the exposure period for a single dose bait is 3 days and the level of efficacy [96-8 (d) (2)] should be 90 % mortality. With the mention of the OPP guideline 1.209, and not providing a copy of the SOP from Bell Laboratories that was followed, it is confusing to know which guidelines are being followed so I revert to the standard 96-8. And, since this is a new bait carrier type, control worms should have been issued to the control group.

4. The field test: The test and control sites were not in the same area, they were "within relative distance of 70 km" (43.5 miles) from each other. There is no guidance in the OPP Pesticide Guideline Subdivision G 96-8 (Mole Toxicants) for distance of complimentary study areas. This seems too far away to be a good representation of test and control subjects for good science. The maps retrieved online make it appear that the control animals were in a much less agricultural setting (urban) and sort of trapped. The treated moles were in a much less restricted area which leads me to think they may have gotten tired of being harassed and just moved off. Since there were only 2 sites to evaluate and no carcasses were retrieved, it is difficult for me to call this 100 %.

5. Even though the assessment holes at both test and control sites were reopened, the treated site had more human activity placing baits as well as a gel worm in the burrow at 1 per meter intervals. The test area was much more condensed and centrally located than the control area which was more spread out across the entire location. These could all be reasons for lack of activity and possible abandonment of the 2 treated assessment holes. The complimentary sites were not treated the same in this respect.

6. According to the Study Outline, the gel worms were manufactured and recieved July 10 to 17, 2003, and analyzed July 11, 2003. The gel worms are labeled at 25 ppm and the analysis shows 19 ppm. How much had the strength of the Bromethalin dropped by the bait day (July 11 to 27, 2003) and did it deteriorate more rapidly when exposed to the elements?

7. Guideline 96-8 (e) (v) states: Verification of the target species. Because the method of censusing is an indirect one, some live trapping before censusing should be done to verify the species of mole and to record the species of mole captured. No mention is made that the species of mole was trapped out to check specifically for eastern moles. Star-nosed moles are also in the same areas in Wisconsin. Positive identification of the species of moles being treated is important because of the species specified on the label for the product.

Conclusion(s) The Bell Laboratory test Experiment #BEL/0803/BE522 is acceptable. More data is needed to make a scientific decision on the fate of the moles in the field trials (Experiment #BEL/0703/BE516) and their identification. The efficacy data submitted to support the claims made for this product are conditonally acceptable.